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RESEARCH ARTICLE

Monitoring introgression in European wildcats in the Swiss Jura

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Abstract Introgression is an important evolutionary force, which can lead to adaptation and speciation on one hand, but on the other hand also to genetic extinction. It is in the latter sense that introgression is a major conservation concern, especially when domestic species reproduce with their rare wild relatives. Hence, monitoring introgression in natural populations subject to hybridization is crucial to elucidate the threat represented by introgression. Here, we monitored introgression between wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*) in a wildcat population in the Swiss Jura Mountains using systematically and non-invasively collected hair samples. We found 21 % admixed individuals based on 68 diagnostic nuclear SNP-markers, corresponding to a migration rate from domestic cats to wildcats of 0.02 migrants per generation. In contrast, gene flow from wildcats into domestic cats was negligible. Haphazard sampling of the same wildcat population, mostly via road kills, led to similar results. Hybridization occurred between wildcat male and domestic cat female and vice versa and, based on the occurrence of backcrosses, both female and male F1-hybrids seem viable and fertile. The observed hybridization pattern may indicate an expanding wildcat population with introgression as a byproduct of this expansion but alternative explanations cannot be excluded with the current data.

Keywords Introgression · Hybridization · Monitoring · Non-invasive systematic sampling · *Felis silvestris*

Introduction

Introgression, defined as the flow of genes between taxa through hybridization beyond the first generation of hybrids, is an important evolutionary force. It can lead to increased genetic variation, adaptation or speciation (Barton 2001; Grant et al. 2004; Mallet 2005; Arnold 2006; Baack and Rieseberg 2007; Mallet 2007). On the other hand, introgression is commonly thought to have a negative effect on rare and endangered species, because it can lead to genetic swamping and extinction (Rhymer and Simberloff 1996; Simberloff 1996; Ellstrand et al. 1999). Introgression is a major concern especially when the source of hybridization is anthropogenic, that is, when domestic or introduced species reproduce with wild or native relatives (crops and wild forms: Ellstrand et al. 1999; dogs and wolves: Randi and Lucchini 2002; cattle and bisons: Halbert and Derr 2007; domestic and wild American mink: Kidd et al. 2009; pigs and wild boars: Goedbloed et al. 2013; sika deer and red deer: Senn and Pemberton 2009). However, introgression of domesticated genes is not necessarily negative. It may introduce genetic variation in the wild population upon which selection can act, facilitating rapid evolutionary changes, as shown in Soay sheep (Feulner et al. 2013). Hence, for conservation purposes, it is crucial to monitor introgression in natural populations and assess the level of threat it represents.

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European wildcats (*Felis silvestris silvestris*) and African wildcats (*Felis silvestris libyca*), have been evolving independently for the last approx. 230,000 years (Driscoll et al. 2007). A low level of natural gene flow between both subspecies, i.e., introgression, may still occur in areas where both subspecies are sympatric. In addition, across much of their range European wildcats face gene flow from the domestic cat, *Felis silvestris catus,* the domesticated form of *Felis silvestris libyca*. Domestication of *Felis silvestris libyca* started at least 9,000 years ago (Vigne et al. 2004). Wild- and domestic cats have potentially been hybridizing for over 2,000 years, since Romans brought domesticated cats into the distribution range of the European wildcat (Faure and Kitchener 2009). At least since the 19th century, the European wildcat populations decreased drastically due to direct persecution and the loss and fragmentation of forested habitat (Schauenberg 1970; Hertwig et al. 2009). At the same time, domestic cats became more popular in Europe and were increasingly bred. In the middle of the 20th century, wildcats were protected by law in several countries, e.g. 1952 in Germany, 1962 in Switzerland, 1976 in France and 1992 in the entire European Union (European Directive 92/43/EEC, Annex IV). Their populations have recovered since then (Nussberger et al. 2007). In parallel, numbers of domestic cats most probably also increased. For example, the Swiss domestic cat population increased from 1.2 to 1.5 million between 1995 and 2012 (Verband für Heimtiernahrung 2013). This increase in density of both cat populations could have favored encounters and thus hybridization between both subspecies.

Wildcats are known to hybridize with domestic cats in several regions (Scotland: Beaumont et al. 2001; Italy: Randi et al. 2001; Hungary: Lecis et al. 2006; Iberic Peninsula: Oliveira et al. 2008a; Germany: Hertwig et al. 2009; France: O'Brien et al. 2009). The Red List of the International Union for Conservation of Nature (IUCN) as well as the Red Lists of several countries mention hybridization with domestic cats as a major threat to the wildcats (BUWAL 1994; ICNB 2004; Haupt et al. 2009; Driscoll and Nowell 2010). Wildcats seem especially threatened through hybridization and genome swamping in Scotland and Hungary, where hybridization rates appear to be very high (Beaumont et al. 2001; Lecis et al. 2006). However, introgression of domestic genes into the wildcat gene pool has been difficult to estimate accurately so far because morphologic and genetic methods failed to reliably recognize hybrids beyond the first generation (Devillard et al. 2013). In addition it is challenging to obtain an unbiased sample of a wildcat population. Sampling from road kills might yield a biased sample because wildcats, hybrids and domestic cats might not be equally often killed on roads. In addition, hybrids with some domestic morphological traits may be underrepresented because they are considered domestic cats by those collecting the road kills and hence not reported. One way to obtain an un-biased sample is with lure stick hair-traps (Kéry et al. 2011). However, genotyping hair samples is challenging due to the highly fragmented DNA and the low DNA copy number resulting in amplification failure of longer PCR products and in allelic dropout (Gagneux et al. 1997; Vigilant 1999). Recent methodological advances alleviate these issues. First, introgression can now be recognized reliably with the development of 187 single nucleotide polymorphism (SNP) markers that are highly differentiated between European wildcats and domestic cats (Nussberger et al. 2013). In addition, a SNP-genotyping method has been optimized to reliably amplify a subset of 96 of these SNP markers from single hairs (Nussberger et al. 2014). Here, we use these SNP-genotyping methods to assess introgression rates in the wildcat population of the Swiss Jura region, based on two contrasting population samples: a non-invasively and systematically collected sample of hair and a haphazardly collected sample set of mostly road kills.

Materials and methods

Sample collection

We had two datasets to estimate introgression in wildcat population of the Swiss Jura: a systematically collected hair sample set (monitoring samples) and a randomly collected sample set (haphazard samples) which originated mostly from road kills.

The monitoring samples were hairs, collected non-invasively by gamekeepers, hunters and ourselves during the winters 2008/09 and 2009/10 using lure stick hair-traps, bated with valerian and sampled every two weeks, five times in total. The hair traps were placed on a regular grid of 5×5 km covering the entire Swiss Jura region (3,719 km^2, 152 sites), the known core distribution range of wildcats in Switzerland (Nussberger et al. 2007) (Fig. 1). The sampling effort was intensified in the cantons of Geneva and Basel-Land to allow a more precise delineation of the western and eastern edges of the wildcat distribution in Switzerland. Sites without forest or within human settlements were excluded, since we expected to find mostly domestic cats in such sites. Three sticks were placed within each site (grid cell of 1 km^2), at least 50 m inside the forest. Hairs from every stick and collecting date were separately collected in 10×15 cm plastic bags (Minigrip) containing a 5 g silicagel Tyvek packet (Dry & Safe GmbH) and stored in a freezer at −80 °C about three days after collection in the field until further analysis.

Haphazard samples, dating from 2000 to 2013, were collected at the Centre for Fish and Wildlife Health of

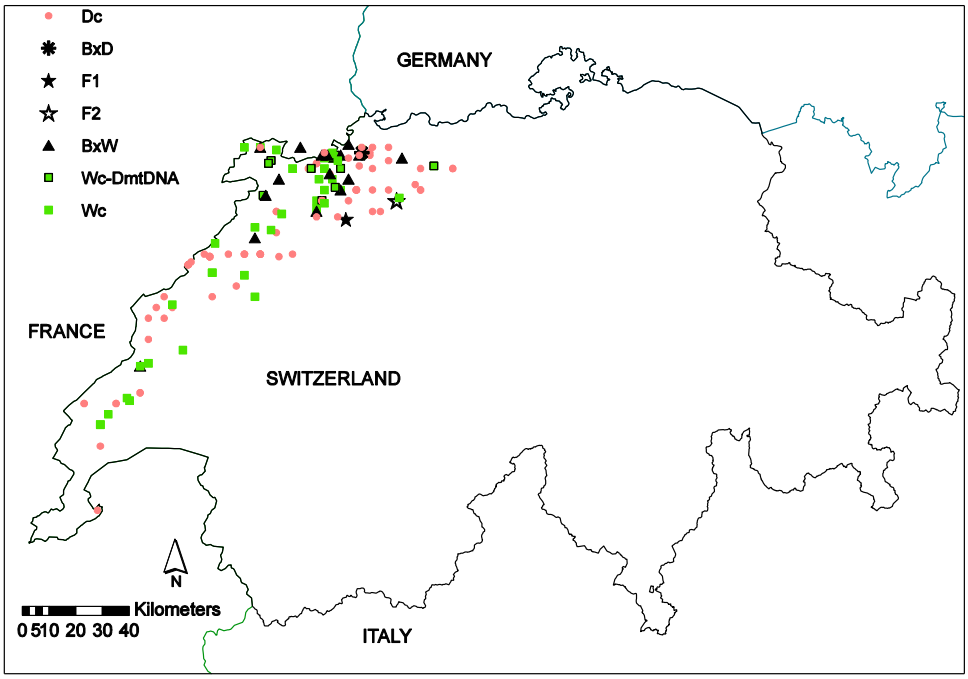


Fig. 1 Sampling sites throughout the Swiss Jura region (monitoring and haphazard samples combined). Wc = wildcat, Wc-DmtDNA = wildcat based on nuclear markers with domestic mitochondrial DNA haplotype, BxW = backcross into wildcat, F1 = first generation hybrid, F2 = offspring of F1 × F1, BxD = backcross into domestic cat, Dc = domestic cat

Berne, Switzerland and several Natural History Museums of Switzerland. The cats were found haphazardly by gamekeepers, hunters and the public, who collected the animals and provided information on the location. This haphazard sample set contained 58 tissue samples and 14 hair samples (**Online Resource 1**) from seventeen cats with domestic morphological features and 55 cats with wildcat morphological features. We used the classic criteria to identify wildcats based on morphology: permanent dorsal line stopping at base of tail, blunt tail tip, distinct tail bands, four stripes on nape, two stripes on shoulder, blurry broken stripes on flanks, rhinarium with upper black margin and gularis with white areola (Ragni and Possenti 1996; Kitchener et al. 2005). Cats were sexed anatomically, where sexual organs were still available.

DNA extraction and quantification

Tissue samples were extracted using the DNeasy Blood & Tissue kit (Qiagen). Hair samples were extracted with the Sample-to-SNP-kit (Applied Biosystems) using a modified protocol (Nussberger et al. 2014). We placed each hair root singly into a 0.2 ml PCR tube, added 9 µl Lysis Solution and placed the tube in a thermocycler at 75 °C for 10 min and 95 °C for 4 min. Finally, we added 9 µl Stabilization Solution.

DNA was quantified using quantitative real-time PCR on a StepOnePlus instrument (Applied Biosystems). PCR contained 2 µl DNA, 10 µl FastStart Universal SYBR Green Master (ROX) 2× (Roche Applied Science), 6.64 µl molecular grade water, 0.16 µl BSA, 0.6 µl forward and 0.6 µl reverse cat specific cMyc primer of 10 µM (F: ACGCACAACGTCTTGGAAC; R: TGGCCTTTTTAAG GATCACC). Initial incubation was set to 10 min at 95 °C, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min (Nussberger et al. 2014). This quantification step enabled us to exclude hair samples with insufficient DNA for accurate genotyping and hairs from other species.

Genotyping

SNP-markers, genotyping assays and method were described in our previous work (Nussberger et al. 2013, 2014) and are summarized in the **Online Resource 2**. Briefly, we genotyped all our samples using 96.96 Fluidigm SNP genotyping arrays (SNP chips). The chip contained 75 autosomal markers with a F_{ST}-value (genetic differentiation index) between wildcats and domestic cats ranging from 0.6 to 1 for introgression diagnosis (75 diagnostic markers), 11 nuclear markers with F_{ST}-values < 0.5 and four mtDNA markers for individual recognition (15 identity markers), four diagnostic mtDNA markers for maternal lineage assessment (four maternal markers) and two diagnostic Y-linked markers for sex determination and paternal lineage assessment (two male markers). Mitochondrial markers were described by Driscoll et al. (2007). We replaced the assay Fst03_SNP149, which was not working in the previous study (Nussberger et al. 2014) by SNP189 (C/T, locus specific primer: GACAATGAGCAAGGCAG GCA, specific target amplification (STA) primer: GTCTAATCAACCCAATACCACCC, allele specific

primer (ASP) 1: ATGATGGCTCGACCAGAAGTTAG, ASP2: ATGATGGCTCGACCAGAAGTTAA). The diagnostic autosomal markers were used to assess introgression and to estimate the migration rate *m* per generation. The two diagnostic markers on the Y-chromosome allowed genetic sexing and assessment of domestic or wild paternal ancestry. The maternal markers were used to assess domestic or wild maternal ancestry. The autosomal markers (diagnostic and identity markers) as well as the mitochondrial markers (identity markers and maternal markers) were used for individual recognition. Since our markers are nearly diagnostic, most individuals are homozygous for most markers and hence we could not test for linkage. In our analysis we assumed they are unlinked.

We only genotyped samples with an estimated DNA concentration of more than 0.005 ng/µl, since below this threshold genotyping usually failed in preliminary tests. For some hair samples, we initially amplified the whole genome (WGA) prior to STA and genotyping, using a single cell WGA kit (WGA4 Sigma Aldrich). However, WGA did not improve hair genotyping success compared to direct STA. Thus, we skipped the WGA for the majority of the hair samples. We used 2, 4 or 10 µl DNA as input quantity into the STA to have at least 50 pg DNA in the reaction. When using 10 µl DNA, we increased the STA-PCR reaction volume to 21 µl, by adding 10 µl Qiagen Master Mix 2× and 1 ul STA-primer mix. These PCR products were diluted 1:5 prior to the SNP-chip genotyping PCR.

We excluded seven diagnostic markers and two identity markers yielding unclear genotyping clusters (Fst01_SNP033; Fst33_SNP152; Fst31_SNP126; Fst37_SNP066; Fst45_SNP153; SNP109; SNP198; ID01_SNP134i; ID07_SNP144i) as well as individual genotypes with more than ten missing values (no calls) for downstream analysis. We also excluded genotypes from monitoring samples which had less than 0.2 ng DNA input, if another hair sample from the same collection bag contained more than 0.2 ng DNA and yielded a similar genotype for the two male markers and the same mtDNA haplotype.

Individual identity assessment for hair samples

To ensure that we did not count a genotype from the same individual multiple times, we assessed identity of each non-invasively collected hair sample using CERVUS 3.0.3 (Kalinowski et al. 2007). Individual genotypes contained 85 markers: 77 autosomal markers (68 diagnostic and nine identity markers) and eight mitochondrial markers (four identity and four maternal markers). We allowed up to 5 mismatches (up to 6 % dissimilar markers) between two genotypes before we attributed them different identities. This threshold was defined based on a preliminary analysis of quality control repeats. This quality control consisted of independently genotyping twice a set of 30 hair samples, 25 that were repeated starting from the STA and five that were repeated starting from the SNP-chip genotyping.

Null alleles

We tested all autosomal loci for null alleles, since they may influence estimates of hybridization (Goodman et al. 1999; Senn and Pemberton 2009). We estimated the correlation between the frequency of non-called samples and the estimated F_{IS} per locus (Beaumont et al. 2001). One would expect null alleles to result in more non-called samples and in more homozygotes relative to expectation and thus in higher F_{IS} values. We tested the domestic cats (and their backcrosses) and wildcats (and their backcrosses) separately. Since the biallelic SNP-markers were chosen to have a very high F_{ST} between these two groups, the rare allele of one group would always be the frequent one in the other group.

Introgression assessment

We assessed introgression between wildcats and domestic cats using Bayesian models without defining a priori pure parental reference samples. For all models, we used individual genotypes containing 68 autosomal diagnostic markers.

First, we assessed each individual's genealogical class using the Bayesian clustering algorithm implemented in NewHybrids 1.1beta (Anderson and Thompson 2002). This framework allows to estimate the posterior probability that each individual of a given sample belongs to each of the following distinct genealogical classes: wildcat (Wc), domestic cat (Dc), first generation hybrid (F1), F2 (offspring of F1 × F1), backcross into wildcat (BxW) and backcross into domestic cat (BxD). The members of a same class have the same expected proportion of per-locus-genotypes with 0,1 or 2 alleles inherited from one taxon. For example, a F1 is expected to be heterozygote at all loci, a BxW is expected to be homozygote for the "wildcat inherited allele" at 50 % of the loci and heterozygote at the remaining 50 % of the loci. These expected proportions are based on Mendelian inheritance patterns. Whether an allele is "wildcat inherited" is estimated from the allele frequencies observed in the data and thus takes into account the uncertainty of the origin of the allele. The main advantage of this method is that one does not need to define a priori pure parental reference samples. With this first model, we identified introgression up to two generations back assuming that individuals were sampled randomly and independently. In addition, a further analysis was performed allowing two more genealogical classes: third

generation backcrosses into Wc (BxWxW) and Dc (BxDxD) respectively. A previous study showed that the markers are powerful enough for distinguishing with an accuracy of over 80 % these third generation hybrid categories (Nussberger et al. 2013). It is very likely that interbreeding between wildcats and domestic cats is much more complex and contains more hybrid categories beyond the third generation. But these hybrids would genetically be quite similar to the parentals. In addition, the number of hybrid categories increases rapidly with every additional generation (number of hybrid categories = $(2^{n-1}+1)(2^{n-1}+2)/2$, where n is the number of generations back; Anderson and Thompson 2002). Thus, parentals and hybrids beyond the fourth generation would be very difficult to identify using the currently available SNP markers.

We reran the default NewHybrids model (six genealogical classes) with the monitoring samples exclusively to estimate the distribution of the π-value, that is, the posterior probability frequency distributions of all the different genealogical classes present in the free-ranging cat population (Anderson & Thompson 2002).

Second, we estimated the membership proportion of each individual to the wildcat and domestic cat populations using the Bayesian clustering algorithm implemented in Structure 2.3.1. (Pritchard et al. 2000). We used an admixture model assuming that there are $k = 2$ populations and applied 100,000 iterations after 10,000 burn-in steps (model of correlated allele frequency, $\lambda = 1$, no prior assumptions about population origin of individuals or alleles). Because we used markers that are highly differentiated among the $k = 2$ populations, relatively few burn-ins and iterations were required to obtain accurate estimates in Structure. Initial analyses revealed that 10,000 burn-in and 100,000 iterations were sufficient and increasing iterations did not yield more accurate estimates (**Online Resource 3**). Structure assigns individuals probabilistically to one or the other population by minimizing Hardy–Weinberg and linkage equilibria in both populations, yielding estimates of the admixture proportion q for each individual. The Structure analysis was repeated five times with different sample seeds. An average over all repeats was calculated. The genotypes of 68 diagnostic markers from the monitoring and haphazard samples were analyzed both independently and pooled.

Third, we inferred the migration rates per generation m, that is, the proportion of gene flow from the domestic cat population into the wildcat population and vice versa, based on 68 diagnostic markers, using BayesAss 3.0.3 (Wilson and Rannala 2003). We inferred the migration rates for each sample set independently and for all samples pooled. The following mixing parameters were applied: migration rates dM = 0.1, allele frequencies dA = 0.2, inbreeding coefficients dF = 0.2, except with the pooled dataset where dA = 0.15 and dF = 0.1. The Monte Carlo Markov Chains were iterated 5,000,000 with a burn-in of 1,000,000 and sampling interval being 1,000.

Results

We collected hair samples in a total of 334 different bags containing between one and 20 hairs from 105 sites. Seventeen sites could not be sampled, whilst at an additional 30 sites, no hairs were found (**Online Resource 4**).

We quantified the nuclear DNA concentration of 669 monitoring hair samples. 159 hairs (24 %) contained more than 0.005 ng/μl and thus qualified for genotyping. The observed low success rate can be explained by the fact that many hairs were likely not from cats and that several cat hairs had degraded roots (in telogen phase; Vigilant 1999), leading to insufficient nuclear DNA extracted from a single hair.

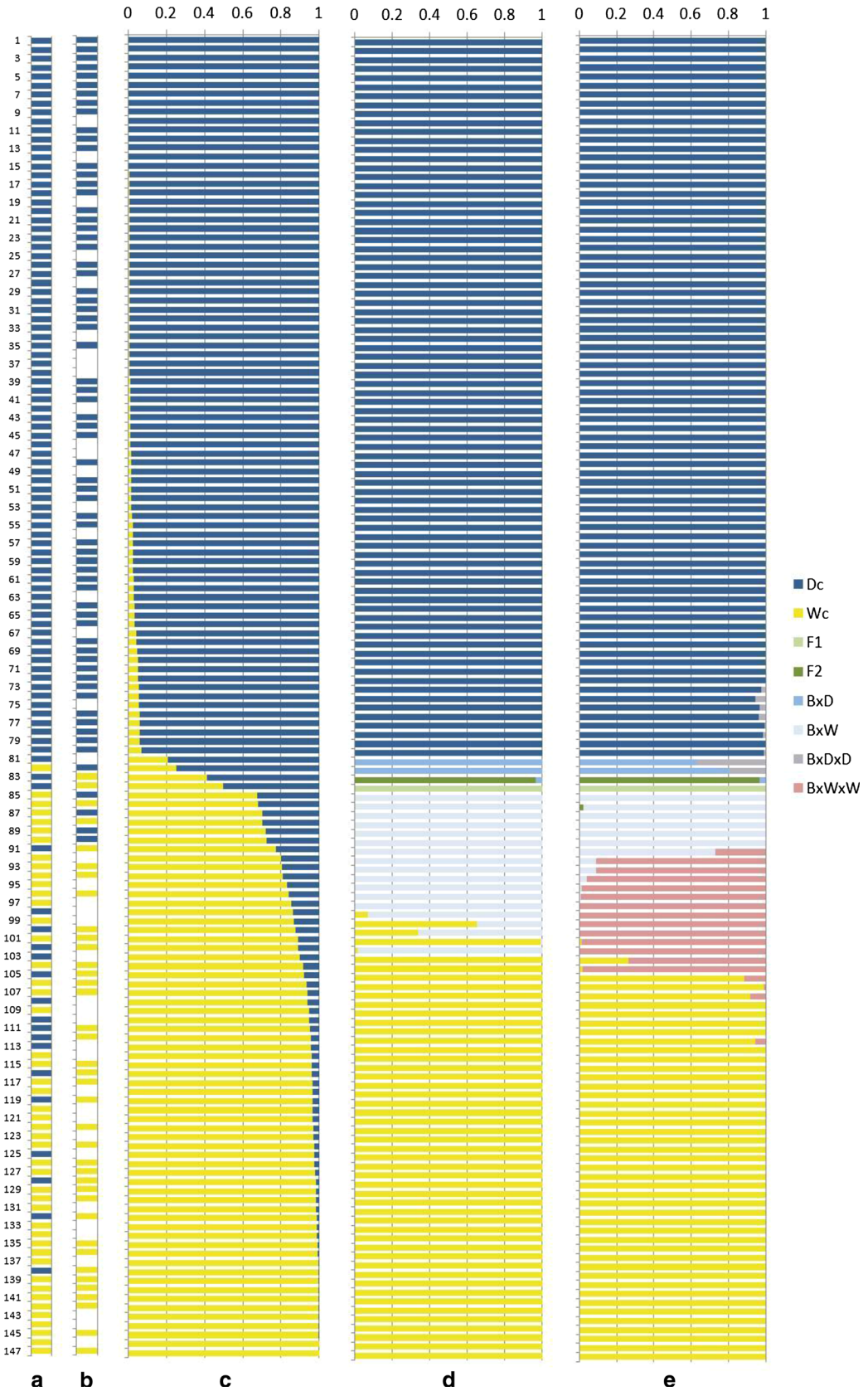
We excluded 22 hairs because they contained more than ten (12 %) values of non-called markers.

Individual identity assessment for monitoring samples

CERVUS identified 75 individuals among the 159 hair samples. Different hairs from the same sampling bag were attributed to a single individual, with only seven exceptions. Six times two domestic cats and one time two wildcats of different sexes left hairs at the same lure-stick at the same collection session. No individual was found at more than one site. Overall, cat samples were found at 49 sites. At most of the sites (34 sites), we only obtained samples from domestic cats, with up to six different individuals at one site. At 13 sites, we detected exclusively wildcats. At four of these 13 sites, two wildcats—mostly one male and one female—were sampled. At the nine other sites, only one wildcat was observed. At the two remaining sites, a domestic cat and a wild- or hybrid cat were simultaneously sampled. Detailed information on all monitoring samples is given in **Online Resource 5** (location, collection day, identity, genotype).

Null alleles

On average, 1.7 % of all single-locus genotypes were not called. The correlation between the frequency of non-called samples and the estimate of F_{IS} per locus was not significant for the wildcat group ($r = 0.017$, $P = 0.998$). The correlation was almost significant for domestic cats ($r = 0.238$, $P = 0.053$), primarily due to a single marker (Fst28_SNP098), for which 22 % of the domestic cats were not called. After removing this marker, the correlation was very low and no longer significant ($r = 0.044$, $P = 0.728$).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Dc
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◀**Fig. 2** Proportion of genome of each individual that is of wildcat ancestry (*yellow*) and domestic cat ancestry (*dark blue*) based on mtDNA (**a**), Y markers (**b**) and nuclear diagnostic markers, as inferred by STRUCTURE (**c**). **d** Posterior probabilities of belonging to the following different genealogical categories based on the NEWHYBRIDS model: domestic cat (Dc, *dark blue*), wildcat (Wc, *yellow*), backcross into Dc (*blue*), F1 (*light green*), F2 (*dark green*), backcross into Wc (*light blue*). **e** same as **d** but with two more classes: third generation backcrosses into Wc (*rose*) and into Dc (*grey*). The horizontal axis gives the proportion of membership to the different categories. For mtDNA and Y markers, only one category (Wc or Dc) per individual is possible, since these haploid markers do not recombine. Every horizontal bar represents one individual. All 147 individuals are sorted in the same order in **a**, **b**, **c**, **d** and **e**

Since the Bayesian analyses returned the same results with and without marker Fst28_SNP098, we retained the locus in the following analyses.

Introgression assessment

The genetic admixture analyses with NEWHYBRIDS and STRUCTURE revealed several individuals with an admixed genome in both sample sets (Fig. 2).

Based on the 68 diagnostic nuclear markers used, NEWHYBRIDS categorized the 75 cats from the monitoring sample as 15 wildcats, four backcrosses into wildcats and 56 domestic cats (all posterior probabilities >99.9 %, Table 1). The frequency distribution of the mixing proportions π of individuals of different genealogical classes indicates that the monitored cat population is composed of roughly 20 % wildcats, 5 % backcrosses into wildcats, 74 % domestic cats and 1 % of the other categories, F1, F2 and backcrosses into domestic cats (Fig. 3). One backcross into wildcat was classified as third generation backcross in the analysis allowing also this additional category. Three of the 15 wildcats carried a domestic mtDNA haplotype. Three out of four backcrosses were males and all three carried a domestic Y chromosome. Overall, 21 % of the sampled individuals of the wildcat population showed signs of recent introgression from the nuclear genome of the domestic cat. When also considering mtDNA introgression, the rate of introgressed individuals increased to 37 %. In contrast, no signs of introgressed wildcat genes were found in the domestic cat population.

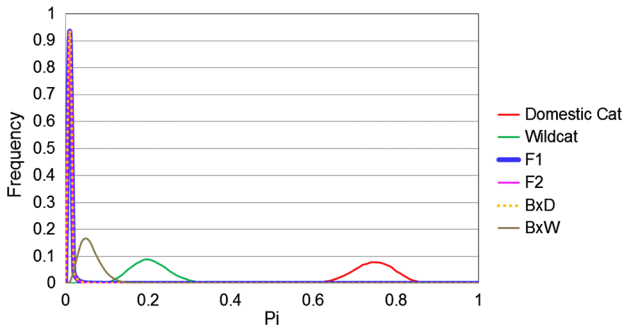

Fig. 3 Proportions of the six genealogical classes (π-value) based on NEWHYBRIDS in the monitoring data set

For the 72 haphazard samples, NEWHYBRIDS detected 34 wildcats (Wc), twelve backcrosses into wildcat (BxW), one F1, one F2, two backcrosses into domestic cats and 24 domestic cats (Table 1). Individuals had over 95 % posterior probability of belonging to their attributed genealogical class with two exceptions. One Wc had a posterior probability of 35 % to be a BxW and one BxW had a posterior probability of 34 % of being a wildcat, suggesting these individuals might be third-generation backcrosses. This was confirmed by the analysis with eight genealogical classes. In that analysis a further eight BxW and two Wc were also re-classified as third generation backcrosses. Twelve of 34 wildcats carried a domestic mtDNA. Hence, 29 % of the wildcat individuals showed signs of introgression, 54 % when including individuals where only the mtDNA showed evidence of introgression. One backcross into wildcat and two-third-generation backcrosses were found in the southern Jura, all other admixed individuals were found in the northern region (Fig. 1). Note, however, that more samples were collected in the northern region.

The migration rate *m* from domestic cats to wildcats per generation was estimated (mean ± SD) as 0.0161 ± 0.0154 individuals per generation in the monitoring sample set, 0.0347 ± 0.0146 in the haphazard sample set and 0.0218 ± 0.0102 when both datasets were pooled (Table 2).

Table 1 Summary of cat samples collected in the Swiss Jura from 2000 to 2013

Sample set	*n*	mtDNA		Y			Nuclear SNPs					
		Wc	Dc	F	Wc	Dc	Wc	Dc	BxW	BxD	F1	F2
Monitoring	75	16	59	13	11	51	15	56	4	0	0	0
Haphazard	72	31	41	29	27	16	32	24	12	2	1	1
Pooled	147	47	100	42	38	67	47	80	16	2	1	1

n number of samples, *mtDNA* number of samples with mitochondrial DNA of wildcat (Wc) or domestic cat (Dc) ancestry, *Y* number of male samples with Y-chromosome of Wc or Dc ancestry (F = number of females), *nuclear SNPs* number of samples classified by NEWHYBRIDS as backcrosss into wildcat (BxW), backcross into domestic cat (BxD), first generation hybrid (F1) or offspring of F1 × F1 mating (F2)

Table 2 Migration rates (m) per generation between domestic cats and wildcats, estimated in BayesAss for monitoring and haphazard sample sets separately and pooled

Sample set	n	m domestic into wild (SD)	m domestic into wild 95 % CI	m wild into domestic (SD)	m wild into domestic 95 % CI
Monitoring	75	0.0161 (0.0154)	0.0000–0.0430	0.0057 (0.0057)	0–0.0169
Haphazard	72	0.0347 (0.0146)	0.0061–0.0633	0.0121 (0.0118)	0–0.0352
Pooled	147	0.0218 (0.0102)	0.0018–0.0418	0.0040 (0.0039)	0–0.0116

n sample size, *SD* standard deviation, *CI* credible interval

The migration rate in the opposite direction was lower in all datasets with values between 0.004 ± 0.0039 and 0.0121 ± 0.0118.

Discussion

We found widespread evidence of introgression from domestic cats into wildcats in the Swiss Jura. Hybridization occurred between wildcat males and domestic cat females as well as between wildcat females and domestic cat males, since we find backcrosses into wildcats with domestic Y or domestic mtDNA. Hence, both female and male F1-hybrids appear to be viable and fertile, at least to some degree. The genetic migration rate was estimated to be about 0.02 domestic migrants per generation into the wildcat population of the Swiss Jura. The strength of the present study resides first in the diagnostic panel of autosomal, Y-linked and mitochondrial SNP-markers, secondly in the reliable genotyping of single hairs, and finally in the systematic sampling of the population in a short time frame based on two independent sampling regimes.

Admixed wildcats

The introgression rate we measured in the Swiss Jura was in the range of the rates observed in most of the surrounding countries of Western Europe. The rate of hybrid wildcats found in our study, ranging from 21 to 54 % depending on sample set and markers used, may seem slightly higher than the rates found in genetic studies of hybridization between wildcats and domestic cats: 14 % were observed in Portugal (Oliveira et al. 2008b), 8 % in Italy (Lecis et al. 2006), 4 % in eastern and 42 % in western Germany (Hertwig et al. 2009) and 36 % in France (Say et al. 2012). For further comparison, 10 % hybrids were found in wild boars (*Sus scrofa*) (Goedbloed et al. 2013) and 4 and 5 % in wolves (*Canis lupus*) in Portugal and Italy, respectively (Verardi et al. 2006; Godinho et al. 2011). However, all these studies applied different genetic markers (e.g. microsatellites) and different hybrid threshold definitions. Some differences in estimated hybridization rates are likely a consequence of these methodological differences. The power of hybridization detection increases when the differentiation of the alleles between the hybridizing taxa increases. In addition, the hybridization rate increases when the threshold of the admixture proportion tolerated before considering an individual as a hybrid is reduced. For example, Oliveira et al. (2008b) and Hertwig et al. (2009) used a wildcat population membership threshold (q_i) of about 0.8 in their STRUCTURE analysis, whereas Say et al. (2012) chose to define their wildcats above a threshold of about 0.9. With these thresholds, not all simulated backcrosses were not recognized as such (e.g. 47 % of simulated backcrosses were classified as pure individuals in the study of Hertwig et al. 2009). In the present study we assessed hybridization rates in wildcats using diagnostic nuclear markers, complemented by mitochondrial and Y-linked markers, and model-based Bayesian methodology that does not rely on defining thresholds. The markers used here outperform the microsatellites used in other studies, because they were deliberately designed for introgression detection. Our marker set correctly recognized simulated backcrosses with more than 99 % accuracy (Nussberger et al. 2013). Thus, more individuals could potentially be recognized as hybrids in our study than in previous ones. This may explain the somewhat higher hybridization rate observed in the Swiss Jura region. However, the percentage of animals with some hybrid ancestry may not be the best way to compare the occurrence of introgression among studies because this measure ignores the degree of hybrid ancestry in each individual. A comparison of the migration rates per generation may be more appropriate to compare the occurrence of introgression among studies. The observed migration rate of 0.02 migrants per generation from domestic cats into wildcats in the Swiss Jura is somewhat lower than the one found in Portugal ($m = 0.064$; Oliveira et al. 2008b) and somewhat higher than in Germany ($m = 0.004$ in Eastern and $m = 0.013$ in Western Germany; Hertwig et al. 2009). This comparison adds to the evidence that introgression rates are not homogeneous over the distribution range of the European wildcat. For example, introgression rates observed in Hungary are almost

four times higher than the ones observed in Italy (Lecis et al. 2006), whilst introgression rates in Scotland are very high (Beaumont et al. 2001).

Gene flow seems to be mostly directed from domestic cats into wildcats rather than in the opposite direction. We only observed two backcrosses into domestic cats in the haphazard dataset (out of 26 domestic cats) and none in the monitoring set (out of 56 domestic cats). Obviously, we might miss some of the backcrosses into domestic cats due to our sampling strategy, which favors wildcat rather than domestic cat sampling (road kill collection mainly if wildcat phenotype; hair collection sites mostly in suitable wildcat habitat). Nevertheless, this bias should apply to backcrossed and pure domestic cats equally and the ratio between backcrossed and pure domestic cats remains very low. Such a directionality in introgression was also observed in Portugal, where migration from domestic into wildcats was $m = 0.064$, while migration from wildcats into domestic cats was a magnitude lower ($m = 0.005$; Oliveira et al. 2008b), and in Western Germany where the estimates were $m = 0.013$ and $m = 0.004$, respectively (Hertwig et al. 2009). In addition, we did not find a wildcat mitochondrial haplotype in any of the domestic cats sampled. In contrast, we found many wildcats having a mitochondrial haplotype clustering with domestic cats. Several processes could explain the asymmetrical gene flow from domestic cats towards wildcats.

First, a wildcat population that is expanding into areas in which domestic cats are already present in higher densities could explain the observed directional introgression. Indeed, at the expansion front, one expects the expanding population to have a lower effective size than the local population. Therefore, the expanding population is expected to have less conspecific gene flow and is prone to get swamped by the genes of the locally well-established population (Currat et al. 2008). In such a scenario, gene flow is directed from the higher density local population (Dc) towards the lower density expanding population (Wc). The theory of Currat et al. (2008) further predicts that genetic markers of the less dispersing sex would be more often introgressed. This results because the less dispersing sex has a lower effective population size compared to the more dispersing sex, resulting in less conspecific gene flow in the less dispersing sex. Our data match this prediction of sexually asymmetric introgression: we observed more introgression of mtDNA markers (18 out of 145 individuals) than of Y markers (4 out of 105 males, $\chi^2 = 4.77$, $P = 0.03$). Moreover, this wildcat expansion hypothesis is congruent with the observation of expanding wildcat populations in France (Say et al. 2012).

Alternatively, the asymmetrical introgression pattern might be explained by mate choice, sex-biased hybrid survival or a combination of both. Indeed, we found several wildcats beyond third generation of hybridization that had mtDNA from the domestic cat, likely indicating ancient introgression of the female line. In contrast, we did not find any domestic introgression of the paternal line going further than the second generation of hybrids. Matings between domestic females and wild males might thus be more frequent than vice versa, or male hybrids with introgressed domestic Y-chromosomes might have a lower survival than hybrids with domestic mtDNA introgression. Such asymmetric hybridization pattern is common. For example, in polecats (*Mustela putorius*) and minks (*Mustela lutreola*), introgression is directed from minks into polecats and matings occur only between male polecats and female minks (Cabria et al. 2011). Moreover, matings between female wolves (*Canis lupus*) and male dogs (*Canis l. familiaris*) seem to be more common than vice versa (Hindrikson et al. 2012 and references therein). Directional and asymmetric introgression patterns were also found between two highly divergent lineages of field voles (*Microtus arvalis;* Beysard et al. 2012).

Geographic distribution of hybrids

We detected hybrids across the Swiss Jura region. However, the ratio of hybrids to wildcats was higher in the northern part of Switzerland (cantons Basel, Solothurn, Jura, Bern: 19 hybrids, 33 wildcats) than in the southwest (cantons of Vaud and Neuchatel: 1 hybrid, 14 wildcats, Fisher's exact test, $P = 0.03$). This suggests that hybridization may be more common in the north than the south. This may be a consequence of the higher human population density in the northern Jura region than in the southern one. Where human density is higher, density of domestic cats may also be higher, leading to increased opportunities for hybrid matings for wildcats.

The Swiss Jura population may represent the edge of a larger wildcat population concentrated in Eastern France. In that case, the hybrids observed in our study may represent a concentration of hybrids at the edge of the wildcat distribution as expected in an expanding population (see above).This is difficult to infer from our data alone, without data from the more central parts of the larger wildcat population. In Italy, wildcat-domestic cat hybrids were found at the periphery of their ecological range (Randi 2008), and the same was found for wolf-dog hybrids in Italy and Portugal (Verardi et al. 2006; Godinho et al. 2011). On the other hand, in France, wildcat-domestic cat hybrids were found throughout the main area of wildcat occurrence (Say et al. 2012). We need to expand sampling to the entire population range (in Switzerland, France, and Germany) to clarify the geographical pattern observed in Switzerland. Also, future monitoring is required to determine whether the geographic pattern of occurrence of hybrids is stable over time, suggesting a hybrid zone

(Shurtliff 2013), or if hybridization is a temporally and geographically variable phenomenon.

Influence of sampling strategies

Achieving unbiased and representative sampling for introgression assessment over a large geographical range is not trivial, and it is important to be aware of possible biases in sampling collection methods. Problematic biases are the ones that are not similar among wildcats, domestic cats and their hybrids, and not quantifiable. Haphazard sampling of road kills is often used (Randi et al. 2001; Verardi et al. 2006; Hertwig et al. 2009), but might be biased for calculating introgression rates: hybrids and backcrosses with a domestic phenotype may be underrepresented in road kill data because they are assumed to be domestic cats and hence not collected, leading to an underestimation of introgression rates from wildcats into domestic cats. On the other hand, hybrids might be overrepresented in such a sample set, if they are more at risk of getting killed on a road, e.g. because they get closer than wildcats to human habitations and thus to denser road networks (Germain et al. 2008; Klar et al. 2008). Another possible bias of haphazard sampling is a spatial one. Road kills may be collected more intensively and less selectively in some areas than others, which could lead to more hybrid discovery and sampling in these areas. These biases can be alleviated, if road kills are exclusively sampled by persons trained to apply the same collection scheme over a given geographical area (Say et al. 2012). It is also possible that some of the biases are smaller than expected. At least in our study, estimates of hybridization rates were similar in both our systematically and non-systematically collected sample sets (21 and 29 %) and the estimated migration rates had largely overlapping credible intervals. Of course, considerations other than bias will often determine the choice of sampling strategy. The advantage of the lure-stick method, for example, is that the intensity and timing of the sampling can be determined by the researcher, which is obviously not possible when relying on traffic killing study subjects.

Implications for conservation

The observed gene flow of 0.02 domestic cat migrants into wildcats per generation may appear relatively low. However, even low introgression can lead to rapid evolutionary changes (Cavalli-Sforza et al. 1994). If we make the—rather unrealistic—assumptions that population size, migration rate and gene flow stay constant over time and that the effect of selection and drift is negligible relative to that of gene flow, a gene flow of 0.02 from domestic cats to wildcats could entirely replace the gene pool of wildcats within 263 generations (or 789 years assuming a cat generation time of three years; see eq. 1.17.2 in Cavalli-Sforza et al. 1994). Nevertheless, several studies showed that introgression is not necessarily bad. For example, introgression may counteract effects of inbreeding, as shown in Darwin's finches (Grant et al. 2003). However, European wildcats do not seem to suffer from high inbreeding at present, since they show a rather high genetic diversity and heterozygosity in Western Europe (Switzerland: Ponta 2012; France: Say et al. 2012). Introgression can also lead to rapid adaptive evolution, as in coyotes (*Canis latrans*) which gained in size through hybridization with wolves (Kays et al. 2010). Hybridization can have positive effects, even when introgressed genes are domesticated, as shown in the Soay sheep (Feulner et al. 2013). In addition, introgression might simply be a byproduct of an expanding population (Petit et al. 2004; Currat et al. 2008). While hybridization may thus not only be negative for wildcats, the substantial levels of introgression detected in this and other wildcat studies, and the very high rates observed in Scotland (Beaumont et al. 2001) and Hungary (Lecis et al. 2006) suggests that introgression remains a major topic in wildcat conservation.

Given that we lack crucial data regarding the consequences of hybridization in wildcats, it is too early for firm management recommendations. Nevertheless, our data suggest that domestic cat spay/neuter campaigns would have to include both males and females since we found that both male and female wildcats hybridize. With our current state of knowledge, it is unclear how effective such campaigns would be. In addition to direct intervention, introgression might be minimized by increasing wildcat population densities through the protection of their habitat against deforestation, fragmentation and disturbance. We hypothesize that a stable or growing wildcat population may better cope with the omnipresent and numerous domestic cats. Such a habitat conservation measure has the additional advantage that it will not only benefit wildcats but also many other species. Most of all, we need a better understanding of the long-term impact of introgression on life-history parameters of wildcat populations.

For conservation purposes, a particularly important question is whether hybrids and introgressed individuals suffer from lower fitness. We did not find any evidence for or against lower hybrid fitness. Several individuals that were wildcats based on the nuclear markers had introgression on mitochondrial DNA. This could be interpreted as evidence that introgressed individuals have been reproducing successfully over many generations. In addition, we observed more than twice as many backcrosses as first generation hybrids. Considering three generations and the pooled data set, we find a ratio of 1:7:13 between first, second and third generation wildcat hybrids. This ratio is not significantly different from the ratio of 1:2:4 we would

expect in the absence of selection and assuming a constant effective population size ($\chi^2 = 1.5833$, $P = 0.4531$). Thus, our data set does not provide evidence that hybrids are under strong selection. However, we stress that such indirect calculations provide only very weak evidence and may well miss major fitness effects on hybrids. What is clearly needed are in-depth studies of hybrids and their fitness in the field. Only such field studies will provide the data necessary to understand the ecological, behavioural and physiological parameters contributing to hybridization, and the demographic consequences of introgression. In addition, systematic wildcat monitoring, based on non-invasive hair sampling and using diagnostic nuclear markers as in this study, will help understand introgression over time and space. Together, this will provide us with a better understanding of the importance of introgression for wildcat conservation.

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Conflict of interest The authors declare no conflict of interest.

References

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229

Arnold ML (2006) Evolution through genetic exchange. Oxford University Press, New York

Baack EJ, Rieseberg LH (2007) A genomic view of introgression and hybrid speciation. Curr Opin Genet Dev 17:513–518

Barton NH (2001) The role of hybridization in evolution. Mol Ecol 10:551–568

Beaumont M, Barratt EM, Gottelli D, Kitchener AC, Daniels MJ, Pritchard JK, Bruford MW (2001) Genetic diversity and introgression in the Scottish wildcat. Mol Ecol 10:319–336

Beysard M, Perrin N, Jaarola M, Heckel G, Vogel P (2012) Asymmetric and differential gene introgression at a contact zone between two highly divergent lineages of field voles (*Microtus agrestis*). J Evol Biol 25:400–408

BUWAL (1994) Rote Listen der gefährdeten Tierarten in der Schweiz. 310.704 d

Cabria MT, Michaux JR, Gomez-Moliner BJ, Skumatov D, Maran T, Fournier P, de Luzuriaga JL, Zardoya R (2011) Bayesian analysis of hybridization and introgression between the endangered european mink (*Mustela lutreola*) and the polecat (*Mustela putorius*). Mol Ecol 20:1176–1190

Cavalli-Sforza LL, Menozzi P, Piazza A (1994) The history and geography of human genes. Princeton University Press, New Jersey, pp 54–59

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. Evolution 62:1908–1920

Devillard S, Jombart T, Léger F, Pontier D, Say L, Ruette S (2013) How reliable are morphological and anatomical characters to distinguish European wildcats, domestic cats and their hybrids in France? *J. Zool. Syst. Evol. Res.* doi:10.1111/jzs.12049

Driscoll C, Nowell K (2010) Felis silvestris. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org

Driscoll CA, Menotti-Raymond M, Roca AL, Hupe K, Johnson WE, Geffen E, Harley EH, Delibes M, Pontier D, Kitchener AC, Yamaguchi N, O'Brien SJ, Macdonald DW (2007) The near eastern origin of cat domestication. Science 317:519–523

Ellstrand NC, Prentice HC, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. Annu Rev Ecol Syst 30:539–563

Faure E, Kitchener AC (2009) An archaeological and historical review of the relationships between felids and people. Anthrozoos 22:221–238

Feulner PGD, Gratten J, Kijas JW, Visscher PM, Pemberton JM, Slate J (2013) Introgression and the fate of domesticated genes in a wild mammal population. Mol Ecol 22(16):4210–4221

Gagneux P, Boesch C, Woodruff DS (1997) Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Mol Ecol 6:861–868

Germain E, Benhamou S, Poulle ML (2008) Spatio-temporal sharing between the European wildcat, the domestic cat and their hybrids. J Zool 276:195–203

Godinho R, Llaneza L, Blanco JC, Lopes S, Alvares F, Garcia EJ, Palacios V, Cortes Y, Talegon J, Ferrand N (2011) Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol Ecol 20:5154–5166

Goedbloed DJ, Megens HJ, Van Hooft P, Herrero-Medrano JM, Lutz W, Alexandri P, Crooijmans RPMA, Groenen M, Van Wieren SE, Ydenberg RC, Prins HHT (2013) Genome-wide single nucleotide polymorphism analysis reveals recent genetic introgression from domestic pigs into Northwest European wild boar populations. Mol Ecol 22:856–866

Goodman SJ, Barton NH, Swanson G, Abernethy K, Pemberton JM (1999) Introgression through rare hybridization: a genetic study of a hybrid zone between red and sika deer (genus *Cervus*) in Argyll, Scotland. Genetics 152:355–371

Grant PR, Grant BR, Keller LF, Markert JA, Petren K (2003) Inbreeding and interbreeding in Darwin's finches. Evolution 57:2911–2916

Grant PR, Grant BR, Markert JA, Keller LF, Petren K (2004) Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. Evolution 58:1588–1599

Halbert ND, Derr JN (2007) A comprehensive evaluation of cattle introgression into US federal bison herds. J Hered 98:1–12

Haupt H, Ludwig G, Gruttke H, Binot-Hafke M, Otto C, Pauly A (2009) Rote Liste gefährdeter Tiere. Pflanzen und Pilze Deutschlands, Bundesamt für Naturschutz, Bonn-Bad Godesberg

Hertwig ST, Schweizer M, Stepanow S, Jungnickel A, Bohle UR, Fischer MS (2009) Regionally high rates of hybridization and introgression in German wildcat populations (*Felis silvestris*, Carnivora, Felidae). J Zool Syst Evol Res 47:283–297

Hindrikson M, Mannil P, Ozolins J, Krzywinski A, Saarma U (2012) Bucking the trend in wolf-dog hybridization: first evidence from Europe of hybridization between female dogs and male wolves. PLoS One 7:12

ICNB (2004) Livro vermelho dos vertebrados de Portugal—Online version. 525-526

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Kays R, Curtis A, Kirchman JJ (2010) Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biol Lett 6:89–93

Kéry M, Gardner B, Stoeckle T, Weber D, Royle JA (2011) Use of spatial capture-recapture modeling and DNA data to estimate densities of elusive animals. Conserv Biol 25:356–364

Kidd AG, Bowman J, LesbarrÈRes D, Schulte-Hostedde AI (2009) Hybridization between escaped domestic and wild American mink (*Neovison vison*). Mol Ecol 18:1175–1186

Kitchener AC, Yamaguchi N, Ward JM, Macdonald DW (2005) A diagnosis for the Scottish wildcat (*Felis silvestris*): a tool for conservation action for a critically-endangered felid. Anim Conserv 8:223–237

Klar N, Fernandez N, Kramer-Schadt S, Herrmann M, Trinzen M, Buttner I, Niemitz C (2008) Habitat selection models for European wildcat conservation. Biol Conserv 141:308–319

Lecis R, Pierpaoli M, Biro ZS, Szemethy L, Ragni B, Vercillo F, Randi E (2006) Bayesian analyses of admixture in wild and domestic cats (*Felis silvestris*) using linked microsatellite loci. Mol Ecol 15:119–131

Mallet J (2005) Hybridization as an invasion of the genome. Trends Ecol Evol 20:229–237

Mallet J (2007) Hybrid speciation. Nature 446:279–283

Nussberger B, Weber D, Hefti-Gautschi B, Lüps P (2007) Neuester Stand des Nachweises und der Verbreitung der Waldkatze (Felis silvestris) in der Schweiz. Mitteilungen der Naturforschenden Gesellschaft in Bern 64:67–80

Nussberger B, Greminger MP, Grossen C, Keller LF, Wandeler P (2013) Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny. Mol Ecol Resour 13:447–460

Nussberger B, Wandeler P, Camenisch G (2014) A SNP chip to detect introgression in wildcats allows accurate genotyping of single hairs. Eur J Wildl Res 60:405–410

O'Brien J, Devillard S, Say L, Vanthomme H, Leger F, Ruette S, Pontier D (2009) Preserving genetic integrity in a hybridising world: are European Wildcats (*Felis silvestris silvestris*) in eastern France distinct from sympatric feral domestic cats? Biodivers Conserv 18:2351–2360

Oliveira R, Godinho R, Randi E, Alves PC (2008a) Hybridization versus conservation: are domestic cats threatening the genetic integrity of wildcats (*Felis silvestris silvestris*) in Iberian Peninsula? Philos Trans R Soc Lond B Biol Sci 363:2953–2961

Oliveira R, Godinho R, Randi E, Ferrand N, Alves PC (2008b) Molecular analysis of hybridisation between wild and domestic cats (Felis silvestris) in Portugal: implications for conservation. Conserv Genet 9:1–11

Petit RJ, Bodenes C, Ducousso A, Roussel G, Kremer A (2004) Hybridization as a mechanism of invasion in oaks. New Phytol 161:151–164

Ponta N (2012) Population structure of wildcats (*Felis silvetris silvestris*) in the Franco-Swiss Jura, as revealed by mitochondrial DNA and microsatellites. Master thesis. Swiss Federal Institute of Technology Zurich and University of Zurich

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Ragni B, Possenti M (1996) Variability of coat-colour and markings system in *Felis silvestris*. Ital J Zool 63:285–292

Randi E (2008) Detecting hybridization between wild species and their domesticated relatives. Mol Ecol 17:285–293

Randi E, Lucchini V (2002) Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conserv Genet 3:31–45

Randi E, Pierpaoli M, Beaumont M, Ragni B, Sforzi A (2001) Genetic identification of wild and domestic cats (*Felis silvestris*) and their hybrids using Bayesian clustering methods. Mol Biol Evol 18:1679–1693

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. Annu Rev Ecol Syst 27:83–109

Say L, Devillard S, Léger F, Pontier D, Ruette S (2012) Distribution and spatial genetic structure of European wildcat in France. Anim Conserv 15:18–27

Schauenberg P (1970) Le chat forestier d'Europe Felis s. silvestris Schreber 1777 en Suisse. Rev Suisse Zool 77:127–160

Senn HV, Pemberton JM (2009) Variable extent of hybridization between invasive sika (*Cervus nippon*) and native red deer (*C. elaphus*) in a small geographical area. Mol Ecol 18:862–876

Shurtliff QR (2013) Mammalian hybrid zones: a review. Mamm Rev 43:1–21

Simberloff D (1996) Hybridization between native and introduced wildlife species: importance for conservation. Wildl Biol 2:143–150

Verardi A, Lucchini V, Randi E (2006) Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. Mol Ecol 15:2845–2855

Verband für Heimtiernahrung (2013) Katzenpopulation 1995–2012 http://www.vhn.ch/daten-und-fakten/zahlen-heimtiermarkt/ Accessed 16 Sept 2013

Vigilant L (1999) An evaluation of techniques for the extraction and amplification of DNA from naturally shed hairs. Biol Chem 380:1329–1331

Vigne JD, Guilaine J, Debue K, Haye L, Gerard P (2004) Early taming of the cat in Cyprus. Science 304:259

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191